4

DETECTING SPECIES BORDERS USING DIVERSE DATA SETS

Examples from Plethodontid Salamanders in California

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1. INTRODUCTION

Debates continue about the appropriate species-level taxonomy to use for plethodontid salamanders. Typically the debates center on what taxonomy is appropriate when geographically contiguous taxa meet and do not become sympatric (e.g., Highton, 1998; Petranka, 1998; Wake and Schneider, 1998), but also at issue is the status of allopatric populations. Differences of opinion are not new to the field of systematics, especially when knowledge is incomplete. What is new, and perhaps surprising, is that these disagreements persist despite the substantial data bases (morphology, allozymes, mtDNA sequences) now available.

Debates over species concepts mask a more general, fundamental agreement that what we call species are ephemeral fragments of a grand evolutionary continuum that constitute a phylogenetic lineage (de Queiroz, 1998). For some workers, the central task is the discovery of clusters of genetically similar populations and the determination of whether such clusters have achieved a particular level of differentiation (e.g., Highton, 2000). The degree to which units hybridize or intergrade with others on their border is a secondary concern. By contrast, other systematists take the position that what is critical is determining when lineages (i.e., ancestral species) have differentiated to the point that they have fallen inexorably apart, thus giving rise to new species (Wake and Schneider, 1998). These workers have adopted Ghiselin's (1997) perspective that

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biological species are populations within which there is, but between which there is not, sufficient cohesion capacity to preclude indefinite divergence. Here information on interactions between differentiated units plays a central role in making taxonomic decisions.

In most cases, there is little or no disagreement. For instance, when completely new kinds of organisms are discovered that are genetically, morphologically, and ecologically distinct (in the strict sense of the meaning of the word, being clearly perceived or marked off), e.g. *Batrachoseps campi* (Marlow et al., 1979) and *B. gabrieli* (Wake, 1996), everybody agrees that they deserve recognition as species. Furthermore, most systematists would accept forms that are morphologically and genetically differentiated as species even when some hybridization occurs (e.g., as between some members of the *Plethodon glutinosus-jordani* complex, Highton and Peabody, 2000).

More problematic are situations in which two sets of populations are perceived to be joined by intermediates. These intermediates might be the result of a pattern of primary geographic differentiation (e.g., Endler, 1977), or of secondary contact between previously isolated and differentiated units. The question is whether the intermediate populations represent a stage in the eventual merger of these groups, or a stable hybrid zone. One would consider all interacting populations to be conspecific if the observer believes there is evidence of sufficient genetic interaction and absence of ecological differentiation that ongoing population interactions will lead to a more or less continuous process of de-differentiation. Alternatively, if a hybrid zone is established that becomes a sink with little or no introgression of genes, one would likely recognize two species unless there was evidence of gene flow between the hybridizing populations by a more indirect route (e.g., a ring species; Wake, 1997). One controversial case is the decision to describe *Plethodon fourchensis*, which intergrades with *Plethodon ouachitae* (Duncan and Highton, 1979). The zone of intergradation is large relative to the small geographic range of "pure" *fourchensis*; accordingly that taxon was synonymized with *ouachitae* by Petranka (1998).

Finally, there are the vexing cases of parapatry and allopatry. One such case in the genus *Rhyacotriton* involved two clusters of populations treated as distinct species, *Rhyacotriton kezeri* and *Rhyacotriton variegatus*, because genetic evidence indicated that the two remained completely distinct where they come into narrow contact even though they resemble each other closely and are not sympatric (Good and Wake, 1992). However, they chose not to describe genetically distinctive groups of populations within *R. variegatus* as separate taxa because in their view fixed genetic differences between putative allopatric or parapatric species show intermediate frequencies in geographically intermediate populations. Their interpretation was that merger of differentiated units was in progress. In contrast, Highton (2000) recommends recognizing additional species, because of the nature of his analytical procedure, which we believe requires him to consider intermediate populations between two differentiated sets of populations to be some kinds of hybrids, especially when the degree of differentiation reaches a certain level which he maintains is general (see extended analysis of hybrid zones and admixture in Wake and Schneider, 1998). In essence, he is making a prediction that units he perceives as distinct are unlikely to continue to merge or admix and will inexorably diverge. In contrast, we accept the empirical evidence of population intermediacy as *prima faciae* evidence of merger in progress and reject the notion of a hybrid zone that, in the case of *R. variegatus*, would be about 100 km in breadth (or using more appropriate measures, minimally on the order of 10^3 home ranges).

In this paper we consider three cases in which discordant patterns of variation lead to problematic taxonomic decisions. These cases are all plethodontid salamanders

from California, the *Ensatina* complex in the Sierra Nevada, and the *pacificus* and *nigriventris* species complexes of *Batrachoseps* in southern California and adjacent parts of Mexico.

2. MATERIALS AND METHODS

We present new data in the form of sequences of the mtDNA gene cytochrome b (cyt b) and allozyme data for *Batrachoseps* in southern California. DNA extraction, PCR and manual sequencing followed the methods of Jackman et al. (1997). Many additional samples were cycle-sequenced using dye-labeled terminators (Perkin Elmer) and separated on an Abi 377 automated sequencer following the manufacturer's directions. Results presented here derive from parsimony analyses of mtDNA from over 200 individuals representing the entire genus (Jockusch and Wake, unpublished), but for the present paper we focus on sequences of 22 individuals from 20 populations throughout the range of *B. nigriventris*, and 84 individuals from 67 localities from the *B. pacificus* complex in southern California. We also present results obtained from previously unpublished allozyme studies for the two complexes of *Batrachoseps* (for allozyme methods and proteins studied see Yanev and Wake, 1981). Distances between mtDNA sequences are reported as Kimura (1980) 2 parameter distances (K2p), calculated in PAUP*4.0d64 (D. Swofford, pers. comm., 1998). Maximum parsimony and bootstrap analyses were conducted using PAUP*4.0d64. For the *B. nigriventris* complex, PAUP*4.0b1 (Swofford, 1998) was used to find the most parsimonious trees under the constraint that *B. nigriventris* is monophyletic. These trees were compared to the most parsimonious trees using Templeton tests (Templeton, 1983). Genetic distances between allozyme samples (D_N) are calculated according to Nei (1972) using BIOSYS-1 (Swofford and Selander, 1981). UPGMA and neighbor-joining (NJ) trees were constructed from the matrices of D_N using MEGA (Kumar et al., 1993). UPGMA trees were used for comparison with Highton (2000). For the *B. nigriventris* complex, allozyme data were also analyzed using the allele coding method of Jackman and Wake (1994) in PAUP*4.0b1.

3. ANALYSIS AND DISCUSSION

3.1. The *Ensatina* Complex in the Sierra Nevada of California

Ensatina eschscholtzii is a classic ring species in a biogeographic sense, in that a series of morphologically differentiated forms have a ring-like distribution around the Central Valley of California, with intergradation or reticulation where forms meet along the axis of the ring but sympatry with hybridization where the ring is crossed (midway in the ring, at the level of San Francisco Bay but in the Sierra Nevada) or closed (at the southern end of the distribution, in inland southern California) (Stebbins, 1949). There are also some weak links and even gaps along the axis of the ring. Stebbins' hypothesis that movement was directional, mainly from the north, has been supported (Jackman and Wake, 1994; Moritz et al., 1992), but the pattern is more complicated than earlier conceived and the migration has not been exclusively southward. A segment of the inner (Sierran) part of the ring was isolated to the south and later rejoined with a northern segment in the central Sierra Nevada (Wake and Schneider, 1998). This reticulation zone, a subject of debate in a taxonomic controversy (Highton, 1998; Wake and

Schneider, 1998), is re-examined in this chapter. We use the taxonomy of Stebbins (1949), treating all taxa as subspecies of *E. eschscholtzii*.

There are several phylogeographic units in the *Ensatina eschscholtzii* complex, recognizable in allozymic data (Jackman and Wake, 1994; Wake, 1997) and in the distribution of mtDNA haplotypes (Wake and Schneider, 1998). Among the inland populations that have a blotched color pattern, there are two main phylogeographic units, a southern lineage that includes the present taxa *klauberi*, *croceater*, and southern populations of *platensis*, and a northern unit that includes northern populations of *platensis*. Between the northern and southern lineages of blotched forms, in the central Sierra Nevada, is a zone of admixture or reticulation (Wake and Schneider, 1998). Just west of Yosemite National Park, a unique mtDNA haplotype clade is found that is part of the the southern lineage. Immediately to the north, only haplotypes of the northern lineage are found. The apparent border is the Stanislaus River. However, this river is not a border from the perspective of allozyme markers, but instead is a region of uniformity. The populations with the unique southern haplotype are variable in allozymes, showing transitions between northern and southern populations. This lack of concordance suggests that the two main haplotype lineages, which are not differentiated with respect to any other markers, are merging (Wake and Schneider, 1998). The decision to place northern and southern *platensis* in the same subspecific taxon constitutes a prediction that merger will continue into the future (there is no reason to expect otherwise). The different patterns of introgression may reflect accidents of history and different patterns in philopatry among the sexes (Wake and Schneider, 1998).

This example illustrates a general pattern in *Ensatina*—wherever subspecies meet along the main axis of the ring, admixture occurs. These subspecies, which are ecologically and morphologically similar but differentiated allozymically or with respect to mtDNA, exchange genes upon meeting (e.g., *Ensatina eschscholtzii oregonensis* and *E. e. xanthoptica* in zones of secondary contact in central coastal California, Wake, 1997). This pattern of reticulation and merging rather than hybridization leads us to conclude that these adjacent, parapatrically distributed units are conspecific. Subspecific taxonomy is appropriate if for convenience one wishes to have names for the differentiated units, which although similar in morphology show subtle but consistent differences in coloration (Stebbins, 1949).

However, secondary contact also has taken place between parts of the ring that are postulated to have been separated sufficiently long to have evolved ecological and morphological differences as well as genetic markers (Wake et al., 1986; Wake et al., 1989). The outcome of these contacts is hybridization (presence of both parental forms as well as first generation hybrids and backcrosses), not admixture, and even sympatry without hybridization at the southernmost location. Hybrids between these distinctive forms are disadvantaged (Wake et al., 1989), whereas there is no hint of disadvantage to individuals in zones of admixture along the axis of the ring. These kinds of interaction between once distant parts of the ring have led some workers to argue for the recognition of two or more species within the *Ensatina* complex (e.g., Frost and Hillis, 1990). There is low tolerance for sympatric subspecies among taxonomists, and such a taxonomy can only be supported if evidence of a ring-like historical biogeography is good. Indeed, many ring species that were once recognized have been broken up by systematists who start at the point of sympatry, taking it as *prima facie* evidence of the existence of two species. But in the case of *Ensatina* the nature of the ring was recognized before any of the instances of sympatry were known and as a result it is an example which has lasted, despite continuing controversy.

We believe that few taxonomists would go so far as to adopt Highton's (1998) proposed taxonomic solution for *Ensatina* (eleven or more species). A less extreme alternative is recognition of the southernmost member of the inland, blotched series, *klauberi*, as a species (a potential solution recognized but rejected by Stebbins, 1949, and Wake and Schneider, 1998; for support of this taxonomy see Frost and Hillis, 1990). This "solution" entails additional problems and does not resolve others. For example, the San Bernardino Mountain populations were identified by Stebbins (1949) as intergrades between *croceater* and *klauberi*; we agree, and believe that they constitute evidence of admixture. We hypothesize an invasion of *croceater*-like populations by *klauberi* moving northward from a southern site of differentiation. If this scenario is correct, *klauberi* is not the end-member of a line of ever-differentiating populations toward the the south (evidence presented by Moritz et al., 1992). This implies secondary contact with admixture, not simply derivation of *klauberi* from a paraphyletic basal unit. Such arguments can be made along the entire axis of the ring (Wake and Schneider, 1998). Furthermore, even if one recognizes *klauberi* as a distinct species, the remaining complex would still be a ring species, because *platensis* and *xanthoptica* are sympatric with very narrow (only two to three home-ranges wide) hybrid zones that appear to be sinks (Wake et al., 1989), thus qualifying for recognition as species using widely accepted taxonomic criteria.

One might continue to partition *Ensatina* until one reaches Highton's (1998) proposal, but even he admitted that data are insufficient to recognize all of his recommended taxa (which have been challenged on other grounds, see Wake and Schneider, 1998). *Ensatina* has resisted extinction, which might well have permanently fragmented the complex had extinction been more extensive. Had those areas in northern California where admixture of blotched and unblotched forms occurs experienced more local extinction, or were blotched populations absent from the San Bernardino Mountains, species borders likely would be apparent.

We continue to support the concept of a ring species for *Ensatina*, as an evolutionary and biogeographic phenomenon, because there is a sense of geographic and genetic continuity, perhaps broken periodically by ephemeral fragmentation and differentiation, but followed by episodes of genetic merger and admixture. Further research on critical areas of postulated admixture may result in more substantive justification for a revised taxonomy than presently exists. Viewed phylogenetically, *Ensatina* is a ring complex, showing many stages in a long, convoluted process of species formation. *Ensatina* illustrates the problem of reconciling process and pattern.

3.2. The Genus *Batrachoseps* in Southern California

While the situation in *Ensatina* is extreme, other complexes of western plethodontids also are difficult to resolve taxonomically. Like *Ensatina*, *Batrachoseps* is distributed in a ring-like pattern around the Central Valley of California (Fig. 1). Because it is a morphologically conservative taxon, insight into its history of fragmentation, differentiation and, in some instances recontact, comes from molecular data.

At present there are 14 recognized species of *Batrachoseps* (Jockusch et al., 1998), but additional species will be described soon. The subgenera (sg) *Plethopsis* (*campi*, *wrighti*) and *Batrachoseps* (remaining species) are well differentiated genetically and anatomically (Jackman et al., 1997). Our current understandings of relationships within the genus as a whole, based on analysis of mtDNA sequence data (Jockusch, 1996, and subsequent studies by Jockusch and Wake, unpublished) is displayed in Fig. 1, and the general distributions of the species groups within California is shown in Fig. 2. Within

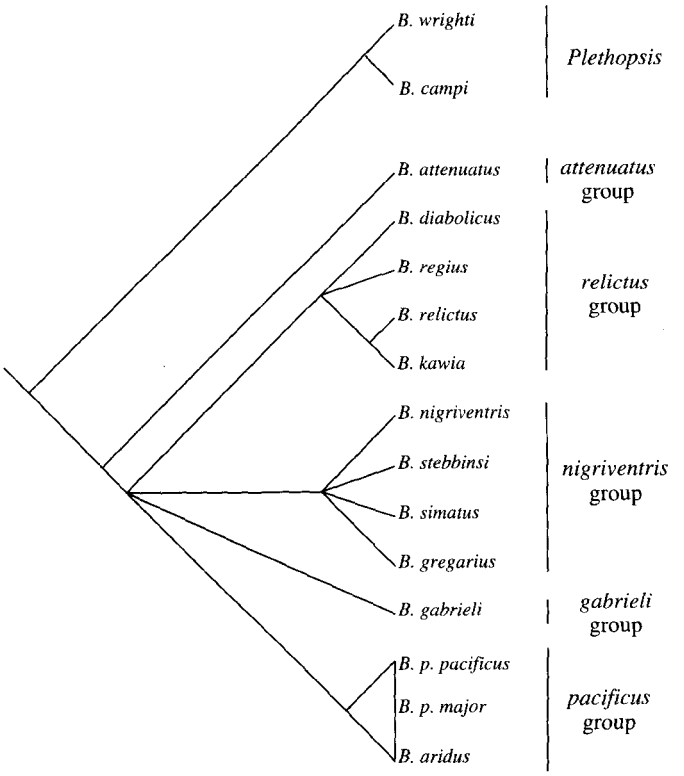

Figure 1. Relationships of the currently recognized taxa in the genus *Batrachoseps* inferred from maximum parsimony analysis of mtDNA sequence data (Jockusch, 1996; Jockusch and Wake, unpublished; details to be presented elsewhere).

sg *Batrachoseps*, we identify five well-supported clades, named by the convention of using the name of the earliest described species contained in each: *nigriventris*, *pacificus*, *relictus*, *attenuatus*, and *gabrieli*. All individuals that have had sequences of cyt b analyzed (more than 200; Jockusch, 1996; Jockusch et al., 1998) fall into these five major clades, and results of allozyme studies (Yanev, 1978; unpublished data) are concordant in the identification of these clades. Sympatry between species belonging to different clades is common, and no evidence of any hybridization exists (contrary to the suggestions of Hendrickson, 1954). We recognize more than one species in each of the first three clades (Jockusch et al., 1998). Only one instance of sympatry is found within any clade, between topotypic *nigriventris* and a population tentatively assigned to *stebbinsi* in the Tehachapi Mountains, southern California).[1] To the south of Ft. Tejon, representatives of three clades occur: *gabrieli*, *pacificus*, and *nigriventris*.

[1] This instance of sympatry may be the earliest recorded in the genus. John Xánthus, who collected the types of *B. nigriventris*, reported in a letter to S. F. Baird (March 1, 1858, reprinted in Zwinger, 1986) "an abundance of salamanders" near Ft. Tejon in the Tehachapi Mountains, and mentioned three species, *Ensatina eschscholtzii croceater* (described as *Plethodon croceater* by Cope, 1867) and two other species, one "smaller, very slender, & of a uniform light brown color" and the other "very small (about 4 inches) very thin, & of a dark brown, or rather sooty black color". These two are likely the two kinds of *Batrachoseps* known to occur in the Ft. Tejon region (where only three species of salamanders are known today), but only two specimens of *B. nigriventris* (the types, Cope, 1969) and none of the second species of *Batrachoseps* were catalogued in the collections of the National Museum.

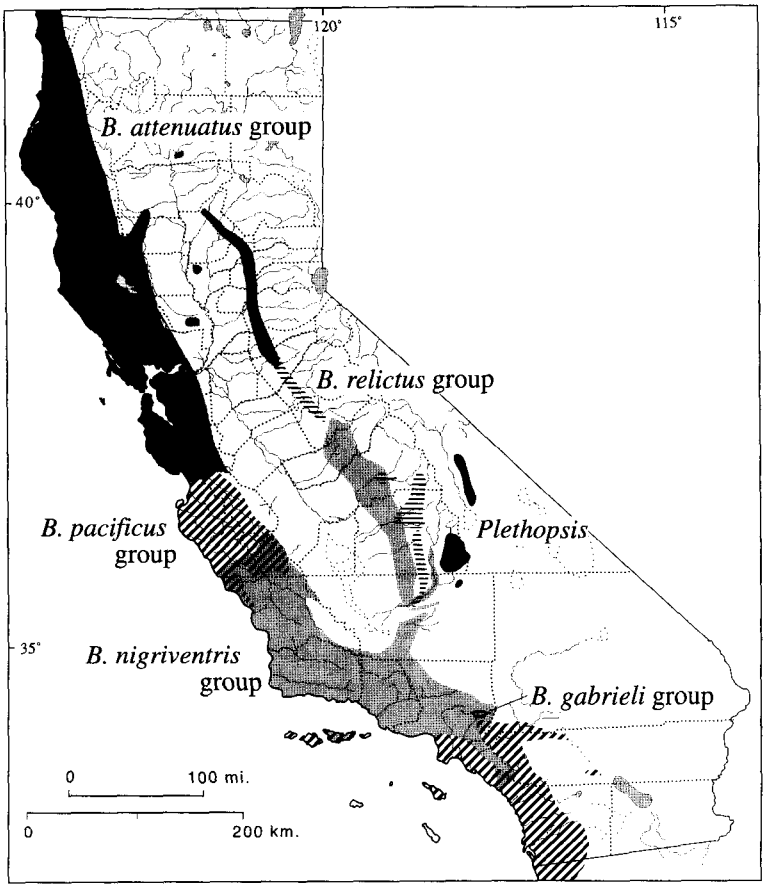

Figure 2. Distribution of the major clades (identified in Fig. 1) of *Batrachoseps* in California. Some mapped distributions for the subgenus *Plethopsis*, and for the *pacificus* group, represent species that are currently undescribed.

Although Campbell (1931) reported two species of *Batrachoseps* in southern California, controversy as to whether one or two species should be recognized persisted until Brame and Murray (1968) diagnosed three species in this region: *pacificus*, restricted to the northern Channel Islands, *major*, on the mainland and on Santa Catalina Island (both in our *pacificus* clade), and *attenuatus* (subsequently southern California populations assigned to this taxon were assigned to *nigriventris* by Yanev, 1980). Here we summarize our findings concerning the *pacificus* and *nigriventris* lineages in southern California, and highlight the history of divergence and reticulation that complicates taxonomic decisions in *Batrachoseps*. We will not discuss the distantly related and recently described *Batrachoseps gabrieli* Wake, 1996, the sole representative of a lineage known only from southern California, nor do we deal with currently undescribed members of the *pacificus* clade isolated well to the north in central coastal California (Fig. 2).

3.2.1. The Batrachoseps pacificus Complex. Yanev (1980) recognized an expanded *B. pacificus* complex that included the taxa *pacificus* and *major* as subspecies, along with *B. relictus* from the Sierra Nevada and unnamed forms from coastal central

Table 1. Samples used in allozyme studies of *Batrachoseps pacificus* group in southern California. Numerals are the same as those in Figs. 3 and 4. Sample sizes indicated in parentheses. Study was conducted on 28 proteins, 26 of which were variable. Samples 1 and 2 used as out-groups.

1. *Batrachoseps gabrieli* from type locality, upper margins of canyon of N Fork San Gabriel River, Los Angeles Co., Ca. ($n = 5$).
2. *Batrachoseps nigriventris* from Limestone Canyon, Orange Co., Ca. ($n = 5$).
3. *Batrachoseps major major* from Irvine, Orange Co., Ca., 33°38.6′N, 117°48.4′W ($n = 10$).
4. *Batrachoseps m. major* from Exposition Blvd at 6th Ave, Los Angeles, Los Angeles Co., Ca., 34°01.3′N, 118°19.3′W ($n = 10$).
5. *Batrachoseps m. major* from Wardlow Canyon, Corona, Riverside Co., Ca., 34°51.6′N, 117°36.8′W ($n = 10$).
6. *Batrachoseps m. major* from Live Oak Canyon, Yucaipa, Riverside Co., Ca., 34°02.2′N, 117°08.2′W ($n = 3$).
7. *Batrachoseps m. major* from Gibbel Rd. at mouth of Avery Canyon, Riverside Co., Ca., 33°42.1′N, 116°56.8′W ($n = 4$).
8. *Batrachoseps major aridus* from Guadalupe Canyon, Riverside Co., Ca., 33°35.3′N, 116°19.8′W ($n = 1$).
9. *Batrachoseps m. major* from Dripping Springs campground, Riverside Co., Ca., 38°27.8′N, 116°59.3′W ($n = 4$).
10. *Batrachoseps m. major* from Will Valley, Palomar region, San Diego Co., Ca., 33°30.5′N, 116°47.8′W ($n = 7$).
11. *Batrachoseps m. major* from Pomo Valley N Ramona, San Diego Co., Ca., 33°05.1′N, 116°51.4′W ($n = 9$).
12. *Batrachoseps m. major* from Solano Hills S San Elijas Lagoon, Solano Beach, San Diego Co., Ca., 33°00.5′N, 117°15.0′W ($n = 4$).
13. *Batrachoseps m. major* from Harbison Canyon, San Diego Co., Ca., 32°50.3′N, 116°48.2′W ($n = 10$).
14. *Batrachoseps m. major* from 27 km W observatory, Pichacho del Diablo, Sierra San Pedro Mártir, Baja California Norte, Mexico, ca. 30°52′N, 115°50′W ($n = 4$).
15. *Batrachoseps m. major* from 1 km N El Rosario, Baja California Norte, Mexico, 30°00′N, 115°30′W ($n = 4$).
16. *Batrachoseps m. major* from Todos Santos Island, Baja California Norte, Mexico, 31°44′N, 116°56′W ($n = 10$).
17. *Batrachoseps m. major* from Santa Catalina Island at Isthmus, Los Angeles Co., Ca., 33°26.5′N, 118°29.3′W ($n = 10$).
18. *Batrachoseps pacificus* from East Anacapa Island, Ventura Co., Ca., 34°00.8′N, 119°22.0′W ($n = 10$).
19. *Batrachoseps pacificus* from Middle Anacapa Island, Ventura Co., Ca., 34°00.2′N, 119°24.6′W ($n = 6$).
20. *Batrachoseps pacificus* from vicinity of Prisoner's Harbor, Santa Cruz Island, Santa Barbara Co., Ca., 34°01.0′N, 119°41.2′W ($n = 5$).
21. *Batrachoseps pacificus* from vicinity of ranch, Santa Rosa Island, Santa Barbara Co., Ca., 33°57′N, 120°05′W ($n = 10$).
22. *Batrachoseps pacificus* from San Miguel Island, Santa Barbara Co., Ca., 34°02′N, 120°21′W ($n = 13$).

California and Baja California. Jockusch et al. (1998) elevated *relictus* to full species rank, thus restricting *pacificus* to the coastal members of Yanev's *pacificus* complex. A close relationship between the taxon *aridus* (described by Brame, 1970), known from two desert sites south and east of Palm Springs (the type locality and a population in Guadalupe Canyon, to the east of the type locality, the latter reported here for the first time) and *pacificus* was first suggested by Yanev (1980). Our work supports this suggestion, as shown below. Here we focus on the southern California members of the *pacificus* complex. We examined allozymes in 20 populations (Table 1, Fig. 3) in southern California and northern Baja California, and we obtained mitochondrial sequences (approximately 750 bp cytochrome b; Jockusch, 1996) for most of these plus many additional populations that have not been studied for allozyme variation (more than 80; Jockusch, 1996, and unpublished information available from the authors).

Results are summarized in Figs. 3, 4, and 5. We expected to find little genetic differentiation in the relatively small geographic area investigated. Results of our allozyme studies showed many frequency differences and a few fixed allelic differences (we do not emphasize these because sample sizes vary and some are relatively small, Table 1). In order to facilitate comparison with the extensive research of Highton

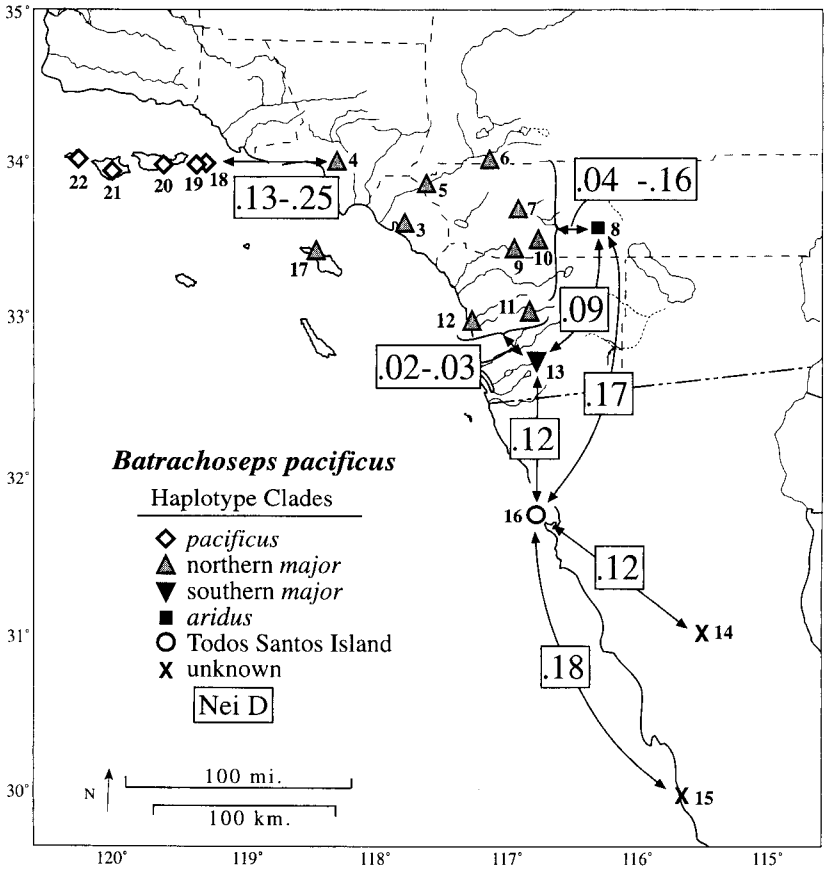

Figure 3. Populations sampled for allozyme analysis of the *pacificus* clade of *Batrachoseps* in southern California. Numbered localities as in Table 1. Boxed values are D_N between units separated by double arrows. Symbols indicate membership in distinctive mtDNA haplotype clades.

(2000), and because we had reason to expect gene flow among populations, we used phenetic analyses. There are two major clusters in the UPGMA analysis of D_N (Fig. 4, Table 2), and these were also found in NJ analysis (not shown). One cluster includes all populations from the northern Channel Islands currently assigned to *B. pacificus pacificus*, while the other includes all mainland populations currently assigned to *B. pacificus major* and the single population (from Guadalupe Canyon) sampled for *B. aridus*. Both island and mainland groups show substantial internal differentiation; maximum divergence within each occurs between geographically more distant populations (to 0.13 in *B. p. pacificus* and 0.26 in *B. p. major*).

Populations of *B. p. pacificus*, a taxon restricted to the northern Channel Islands, are morphologically differentiated from mainland *major* (Brame and Murray, 1968), and the taxa are also well differentiated with respect to allozymes. D_N to northern *major* is from 0.13–0.25. There are no fixed differences separating the two groups, but there is one nearly fixed difference (for alcohol dehydrogenase 2) and *pacificus* has a few allozymic variants not found on the mainland. There is substantial allozymic variation within *pacificus* (D_N reaches 0.13 between populations from the easternmost and

Table 2. Values of D_N between populations of the *Batrachoseps pacificus* complex in southern California, as well as for two outgroups.

Population	1	2	3	4	5	6	7	8	9
1 *gabrieli*									
2 *nigriventris*	0.748								
3 Irvine	0.834	0.371							
4 Los Angeles	0.692	0.405	0.074						
5 Corona	1.001	0.504	0.114	0.160					
6 Live Oak Canyon	0.981	0.465	0.062	0.129	0.062				
7 Avery	0.838	0.432	0.098	0.160	0.085	0.059			
8 *aridus*	0.862	0.377	0.035	0.088	0.119	0.060	0.092		
9 Dripping Springs	0.857	0.428	0.103	0.146	0.147	0.124	0.110	0.157	
10 Mt. Palomar	0.683	0.354	0.040	0.075	0.153	0.103	0.121	0.058	0.077
11 Pomo Valley	0.750	0.344	0.063	0.110	0.147	0.111	0.108	0.089	0.051
12 Solano Hills	0.711	0.399	0.047	0.097	0.154	0.108	0.114	0.069	0.108
13 Harbison Canyon	0.755	0.358	0.056	0.103	0.144	0.102	0.106	0.089	0.051
14 S. San Pedro Martir	0.733	0.410	0.122	0.147	0.234	0.207	0.227	0.134	0.149
15 El Rosario	0.699	0.516	0.141	0.098	0.228	0.173	0.214	0.127	0.256
16 Todos Santos	0.656	0.430	0.136	0.158	0.247	0.207	0.239	0.170	0.165
17 Catalina	0.879	0.377	0.017	0.089	0.127	0.087	0.117	0.061	0.116
18 East Anacapa Is.	0.804	0.474	0.160	0.198	0.219	0.217	0.147	0.173	0.227
19 Mid Anacapa Is.	0.733	0.428	0.208	0.246	0.270	0.266	0.178	0.220	0.277
20 Santa Cruz Is.	0.727	0.346	0.148	0.185	0.252	0.206	0.127	0.160	0.213
21 Santa Rosa Is.	0.713	0.370	0.146	0.185	0.251	0.202	0.126	0.158	0.227
22 San Miguel Is.	0.790	0.455	0.215	0.257	0.307	0.264	0.188	0.231	0.290

westernmost islands). In addition to the allozyme differences, *pacificus* has unique (but variable) mtDNA haplotypes that form a clade well separated from all others (Fig. 4).

While allozyme and mtDNA data concur on the distinctiveness of *pacificus*, elsewhere they give conflicting pictures of the history of the *pacificus* complex (Figs. 4 and 5). In the mtDNA tree, part of *B. p. major* (which we henceforth identify as northern *major*) forms a clade with two mtDNA lineages found more than 300 km northwest of the closest population of major (Jockusch, 1996). These populations occur near the Monterey-San Luis Obispo county line in the central Coast Ranges and were included with populations found still farther north as an undescribed subspecies of *pacificus* on the basis of allozyme data by Yanev (1980). Northern *major* occupies most of the range of *major* as a whole, except for central San Diego County and areas to the south. The sister group of northern *major* plus the central Coast Range populations is a clade containing four geographically separated lineages: *pacificus* (*sensu stricto*, on the northern Channel Islands), *aridus* (including both topotypic and Guadalupe Canyon populations), a group of populations from central and southern San Diego County (which we call southern *major*), and a population from Todos Santos Island, off the northwestern coast of Baja California. No other sequences are available from populations in Baja California. The *aridus* and Todos Santos haplotypes are well differentiated (about 5% diverged) from each other but are sisters in phylogenetic analysis, and this clade is likely sister to southern *major* (Fig. 5). The taxon currently known as *B. pacificus major* is not monophyletic with respect to its mitochondrial DNA. Northern and southern *major*, which we are unable to distinguish morphologically or allozymically (Fig. 3, see also below), are parapatrically distributed in San Diego County, with northern *major*

10	11	12	13	14	15	16	17	18	19	20	21
0.024											
0.031	0.051										
0.025	0.022	0.049									
0.076	0.112	0.070	0.100								
0.142	0.180	0.114	0.187	0.183							
0.085	0.109	0.099	0.119	0.118	0.178						
0.071	0.069	0.073	0.089	0.154	0.167	0.153					
0.181	0.163	0.163	0.185	0.282	0.290	0.280	0.142				
0.229	0.210	0.212	0.233	0.337	0.340	0.324	0.188	0.043			
0.167	0.151	0.153	0.171	0.267	0.279	0.265	0.129	0.074	0.037		
0.169	0.163	0.149	0.178	0.270	0.275	0.271	0.133	0.083	0.046	0.005	
0.238	0.211	0.215	0.242	0.350	0.354	0.333	0.186	0.127	0.103	0.063	0.054

extending nearly to the southern border of the county immediately adjacent to the Pacific Coast. The two haplotypes, differing by about 8%, have been found in sympatry in the vicinity of Mt. Gower in northern San Diego County, and near sympatry at several sites in the city of San Diego.

The most striking results of our study of the *pacificus* complex relate to differences in our two data sets with respect to *major* and *aridus* (Tables 2 and 3). In the points of conflict each data set supports a different resolution, and does not support the alternative history inferred from the other data set. Accordingly, these differences likely represent real differences in the underlying history of mtDNA and allozymes. In the mtDNA data, we find two distinct lineages that are not phylogenetic sisters within current *major*, whereas two groups are not recognizable in our allozyme data. The lowest D_N within *major* is only 0.02, a level typical of neighboring conspecific populations, recorded between two populations (11 and 13, Table 1) in San Diego County having northern and southern mtDNA, respectively. The lack of allozymic differentiation of *aridus* (D_N to 11 populations of northern and southern *major* in southern California ranges from 0.04–0.16, $\bar{x} = 0.08$) is remarkable given its morphological and ecological divergence from other southern California populations (for comparison, D_N among 11 populations of *major* in southern California, including the Santa Catalina island sample, ranges from 0.02–0.16, $\bar{x} = 0.09$). Although the Todos Santos population is neither morphologically nor ecologically differentiated relative to *major*, it has mtDNA as distinctive as that of *aridus*. The Todos Santos population has a value of D_N to *aridus* of 0.17, and an average value of D_N to southern California *major* of 0.16 (range of 0.09–0.25). Values of D_N within *major* in southern California are as high as 0.16, but reach values of 0.23 and 0.26 if two populations at the extreme periphery of the range

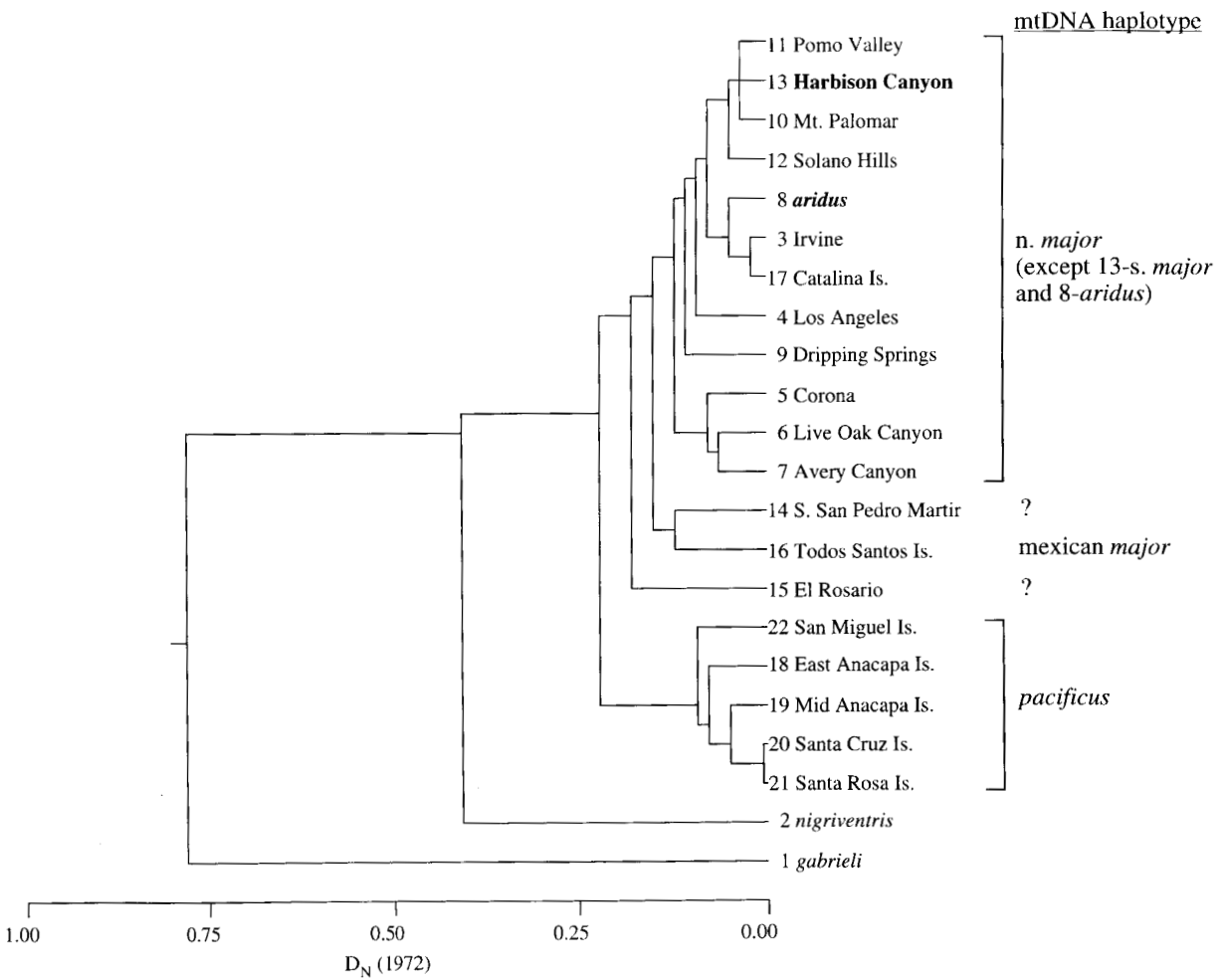

Figure 4. UPGMA tree derived from matrix of D_N (Table 2) for samples of the *Batrachoseps pacificus* complex in southern California and outgroup taxa. Column on right indicates affiliation based on mtDNA haplotype. Bold faced localities are exceptions (Harbison Canyon is southern *major*) within a cluster otherwise containing only northern *major* haplotypes. Question marks indicate that haplotypes are unknown.

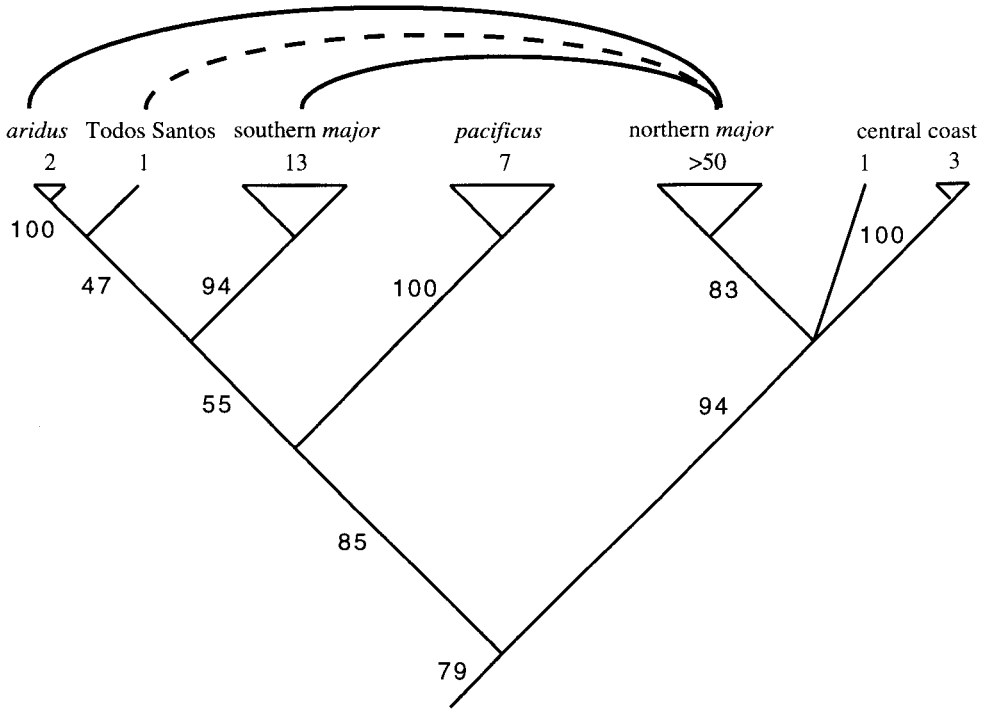

Figure 5. Relationships of mtDNA haplotypes for the *pacificus* clade of *Batrachoseps* in southern California based on maximum parsimony analysis. Significant clades are named. Numbers below names indicate the number of individuals sampled. Numbers along branches are percent of 100 bootstrap replicates in which that clade was supported (Jockusch and Wake, unpublished analyses). Solid lines above tree join mtDNA clades with minimum $D_N < 0.05$ between some populations. Dotted line joins mtDNA clades with minimum $D_N < 0.10$ between some populations.

in Baja California are included (unfortunately we have been unable to obtain mtDNA sequences from these Baja California samples).

We hypothesize that the mtDNA pattern reflects an early geographic fragmentation in southern California and the subsequent differentiation of isolates. The presence of related haplotype clades on the geographic fringes of the most widely distributed clade suggests that the geographical isolates might have been displaced from or replaced in the more central areas by an expanding northern *major*. Lack of allozyme differentiation across the mtDNA contact zone between northern and southern *major* argues that these populations are not reproductively isolated and are in the process of merging. Evidence of some admixture of *major* with *aridus* and the population on Todos Santos Island is seen in the pattern of shared proteins. In consequence, the latter two populations cluster within *major* in both the UPGMA and NJ analyses of allozyme data.

Salamanders of the genus *Batrachoseps* are sedentary (Cunningham, 1960; Hendrickson, 1954). In plethodontids, male salamanders move more than females (e.g., Staub et al., 1995), and the geographically restricted distributions of mtDNA haplotypes (Jockusch, 1996) show that female movement is limited in *Batrachoseps*. Accordingly, it makes sense that remnants of the divergent, maternally-inherited mtDNA haplotypes persist in peripheral populations. The geological history of

Table 3. Allozyme and mtDNA divergence between mtDNA haplotype groups in southern members of the *pacificus* complex of *Batrachoseps*. Numbers above the diagonal are range of K2p distances. Numbers below the diagonal are range of D_N. Along the diagonal (in boxes) are the average K2p distances for the basal split within the mtDNA clade (top) and maximum D_N (bottom) found within each group—indicating that only a single population was sampled. n.a. indicates that those populations were not compared in a single allozyme study. Localities for central coast populations are Pine Mountain, San Luis Obispo Co. (allozymes and mtDNA), Hwy. 1, 0.4 mi N. Monterey-San Luis Obispo county line, Monterey Co. (mtDNA), Santa Rita Old Creek Road, San Luis Obispo Co. (allozymes and mtDNA), and York Mountain Winery, San Luis Obispo Co. (allozymes).

	aridus	Todos Santos	s. *major*	*pacificus*	n. *major*	Central coast
aridus	0.005	0.043–0.056	0.051–0.073	0.051–0.080	0.074–0.113	0.079–0.111
	—					
Todos Santos	0.19	0	0.045–0.056	0.047–0.062	0.067–0.097	0.081–0.090
		—				
southern *major*	0.09	0.12	0.037	0.051–0.084	0.064–0.107	0.087–0.108
			—			
pacificus	0.16–0.23	0.27–0.33	0.17–0.24	0.025–0.13	0.058–0.100	0.078–0.099
northern *major*	0.04–0.16	0.09–0.25	0.02–0.14	0.13–0.31	0.041–0.16	0.052–0.096
central coast	n.a.	n.a.	n.a.	0.17–0.29[1,2]	0.20–0.28[1,2]	0.069–0.19[1]

[1]Data from Yanev (1978).

[2]Lower distances occur to other populations in the central Coast Ranges treated by Yanev as an undescribed subspecies of *B. pacificus*.

southern California is complex, for the area is extremely active tectonically and its paleogeography has yet to be fully reconstructed (Atwater, 1989). Sharp climatic shifts related to rain shadows, mountain barriers, and topographic factors likely contributed to the paleoecological diversity of the region and promoted isolation and local differentiation. While our evolutionary scenario may appear to be relatively complex, we nevertheless believe it to be the simplest interpretation of the data. In our view, a once-widespread ancestral form began to fall apart as a genetically cohesive unit, but after differentiating to various degrees some parts have come back together. In the process, merger at the level of nuclear genes (as reflected in our allozyme data) occurred, but this merger has thus far not wiped out the deeper history which persists in the signal given by remnant mtDNA haplotypes.

What can happen with even short periods of isolation, or a little targeted extinction, is shown by *aridus*, which although only slightly differentiated with respect to allozymes is distinctive morphologically. Currently *aridus* is isolated in two populations surrounded by desert habitat unsuitable for salamanders. However, the nearest population of *major* is found just 28 km to the northwest, and the intervening habitat was likely more suitable for salamanders at various times in the late Pleistocene than at present (Van Devander and Spaulding, 1979). Present-day *aridus* has retained its distinctive mtDNA but likely has interbred with northern *major* in the recent past, as reflected in its close allozymic similarity to that taxon (D_N is as low as 0.04 from *aridus* to populations of northern *major*; *aridus* has no alleles that are not also found in

northern *major*, but we have only had a single specimen of *aridus* for electrophoretic studies). The distinctive ecology and morphology of *aridus* might be a result of long sustained selection that produced adaptive changes that survived the postulated admixture with northern *major*. For example, the relatively broad head and short tail of *aridus* have the effect of reducing the surface:body volume ratio, and this might be of significance in reducing water loss in these desert-dwelling forms. Alternatively, *aridus* may have diverged morphologically and ecologically only recently and simply retains an ancient haplotype that has been geographically marginalized. Phenotypic plasticity (documented in *Batrachoseps*; Jockusch, 1997) may have contributed to rapid morphological differentiation of *aridus*.

Taxonomy of the *pacificus* group has been unstable. At present the populations considered here are treated as two subspecies of *pacificus* and a separate species *aridus*. The largest unit that one might recognize as a single species would include all of the above as well as unnamed lineages along the central California coast and in Baja California (which are all currently included in *pacificus*, Stebbins, 1985). Such a classification seems to us to be excessively conservative. In particular, we believe that the central coastal forms merit recognition as distinct species (we will deal with these in a separate paper).

The most radical taxonomy would be to use the mtDNA tree as a guide to recognition of monophyletic mtDNA lineages as taxa, and in the absence of allozyme data this taxonomy likely would be adopted. Thus, *pacificus*, northern *major*, southern *major*, *aridus*, and the Todos Santos populations would all be recognized as separate species (it would take additional research to determine if either southern *major* or the Todos Santos population might be called *leucopus* Dunn 1922, type locality Coronados Islands, 80 km NW of Todos Santos; alternatively, northern *major* is geographically closest to the Coronados Islands and the populations on those islands might have its mtDNA). The Coronados Islands and mainland Baja California populations would be left in an uncertain status pending study of their mtDNA. However, because the allozyme data provide strong evidence that northern and southern *major* are in the process of admixing, in essence reversing the phylogenetic course suggested by the mtDNA phylogenetic hypothesis, we reject this taxonomic solution. This highlights a pitfall in drawing taxonomic conclusions based on mtDNA data alone.

There are several alternatives between the two extreme taxonomies outlined above. One possibility would be to recognize *pacificus*, *aridus*, and *major* at the specific level. The morphologically distinctive (Brame and Murray, 1968) populations on the northern Channel Islands appear to be on an independent evolutionary trajectory and are distinctive in allozymes and mtDNA. This taxon would take the name *pacificus*, and the southern California mainland and Santa Catalina Island populations would take the name *major*. The forms along the central coast would be placed in at least two, and possibly three or four, species. They are diagnosable, independently evolving lineages based on their morphological traits and evidence of long separation from their southern California relatives is seen in the large amount of genetic differentiation displayed. The status of *aridus* would be problematic. Although no-one has previously contested its species status, we have presented the allozymic data that cause us to question its validity. Furthermore, while the northern Channel Islands have been long-separated from the mainland and the central coastal forms are separated from the southern California species by hundreds of kilometers, the current isolation of *aridus* from other southern California populations is both narrow and probably ephemeral.

We choose a classification that recognizes only two species in the *pacificus* group

in southern California, *pacificus* on the northern Channel Islands and *major* everywhere else, and reduces *aridus* to a subspecies of *major*. This classification recognizes the unique morphology and evolutionary history of *aridus*, but conveys genealogical relationships as we presently understand them. It is widely acknowledged that species can be paraphyletic, if for example a peripheral population of a widespread species diverges quickly and is recognized as a species, rendering the remnant paraphyletic. At the infraspecific level, the possibilities of paraphyly are increased. For example, within *Ensatina* the subspecies *platensis* is recognized as having two widely divergent mtDNA lineages, but its recognition as a single taxon is supported by Wake and Schneider (1998) on the grounds that it forms a morphologically distinct cluster of populations set off by differences in allozymes and mtDNA from other such clusters. In general we do not support the wide use of subspecies. Phylogeographic patterns for mtDNA haplotypes can readily be presented and expressed without formal taxonomic names. But when geographically restricted groups of populations that share distinguishing morphological, ecological, or behavioral traits are identified that show evidence of merger with other such groups, such as in the case of *aridus*, we believe that there is a place for trinomials. The classification we favor is as follows:

3.2.1.1. *Batrachoseps pacificus* Cope 1865. Distribution: East Anacapa, Middle Anacapa, West Anacapa, Santa Cruz, Santa Rosa and San Miguel Islands, California.

3.2.1.2. *Batrachoseps major* Camp 1915. *Batrachoseps major major* Camp 1915

Distribution: Southern California mainland from the southern foothills of the Santa Monica, San Gabriel and San Bernardino mountains south to the western slopes of the Sierra San Pedro Mártir in Baja California and along the Pacific Coast as far south as El Rosario (30° N); entering the southern California desert through San Gorgonio Pass and occurring south of Cabazon, in Snow Creek Canyon, and in the city of Palm Springs; on Santa Catalina, Coronados and Todos Santos islands. The following taxa are considered to be subjective junior synonyms: *catalinae* Dunn 1922; *leucopus* Dunn 1922. This taxon includes the population from the Sierra San Pedro Mártir treated by Yanev (1980) as an undescribed subspecies of *B. pacificus*, but not recognized taxonomically by us. Elevational range is from sea level to at least 2330 m in the Sierra San Pedro Mártir (Mahrdt et al., 1998), but the species is not known above 1500 m (on Mt. Palomar, San Diego County) in the United States.

Batrachoseps major aridus Brame 1970

Distribution: Hidden Palm Canyon and Guadalupe Canyon, in the northern slopes of the Santa Rosa Mountains, Riverside County, California. Elevational range is about 760–1000 m.

3.2.2. The Batrachoseps nigriventris Complex. Although *B. nigriventris* was described early (Cope, 1869, type locality Ft. Tejon, Kern County) it was considered to be a synonym of *attenuatus* until Yanev (1980) showed that it was differentiated in allozymes and clustered more closely with other taxa than with *attenuatus*. Yanev's *nigriventris* extended from the foothills of the central and southern Sierra Nevada and from the central Coast Range south through the Tehachapi and San Gabriel Mountains into southern California. Yanev also included populations from Santa Cruz Island (where they are sympatric with *pacificus*) in her *nigriventris*. Populations from the Sierra Nevada and its western slopes recently were described as a distinct taxon, *Batrachoseps gregarius* Jockusch, Wake and Yanev 1998. There is substantial mtDNA

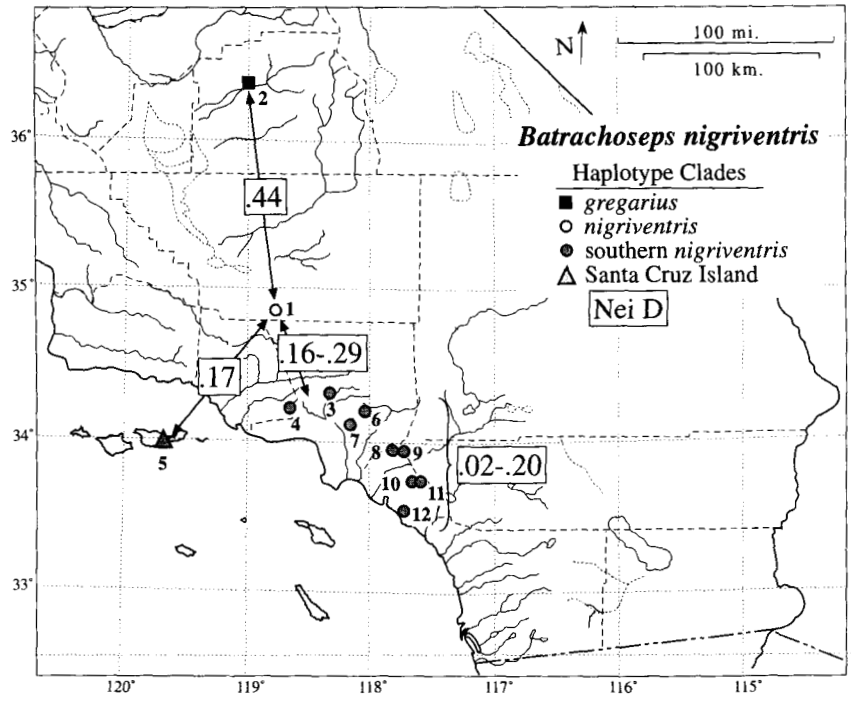

Figure 6. Populations sampled for allozyme analysis of the *nigriventris* clade of *Batrachoseps* in southern California. Numbered localities as in Table 2. Boxed values are D_N between units separated by double arrows. Symbols indicate membership in distinctive mtDNA haplotype clades.

haplotype differentiation among the remaining constituents of *nigriventris*, with three discrete lineages represented: (1) northern *nigriventris* from the vicinity of the type locality west and north to the periphery of its range in southern Monterey County, (2) Santa Cruz Island populations, and (3) southern California populations distributed from east-central Ventura County and northwestern and western Los Angeles County to the southern and eastern periphery of the species range (Fig 6). For purposes of this paper we refer to this last set of populations as southern *nigriventris*.

The concordance between the allozyme and mtDNA data sets for the *nigriventris* complex is high with respect to identification of lineages (Figs. 6, 7, and 8). As was the case in the *pacificus* complex, the northern island population is distinct in both mtDNA and allozymes (Nei D = 0.17 between it and topotypic *nigriventris*; we lack a direct allozymic comparison of this population to southern *nigriventris* populations). Populations studied are listed in Table 4 and mapped in Fig. 6. Both mtDNA and allozyme data show that southern and northern *nigriventris* have diverged substantially. In our analysis of the mtDNA data, they do not appear to be sister taxa; rather southern and island *nigriventris* are sister lineages (Fig. 8). Furthermore, these two lineages are more closely related to those of *stebbinsi* and *simatus* (included within "other lineages", Fig. 8) than to northern *nigriventris*. Accordingly, one might conclude that southern *nigriventris* also merits description as a distinct species. There is surprisingly large allozymic divergence within southern *nigriventris* (D_N to 0.20; Table 5), and while there is only a single lineage of haplotypes, it also displays substantial differentiation (Table 6).

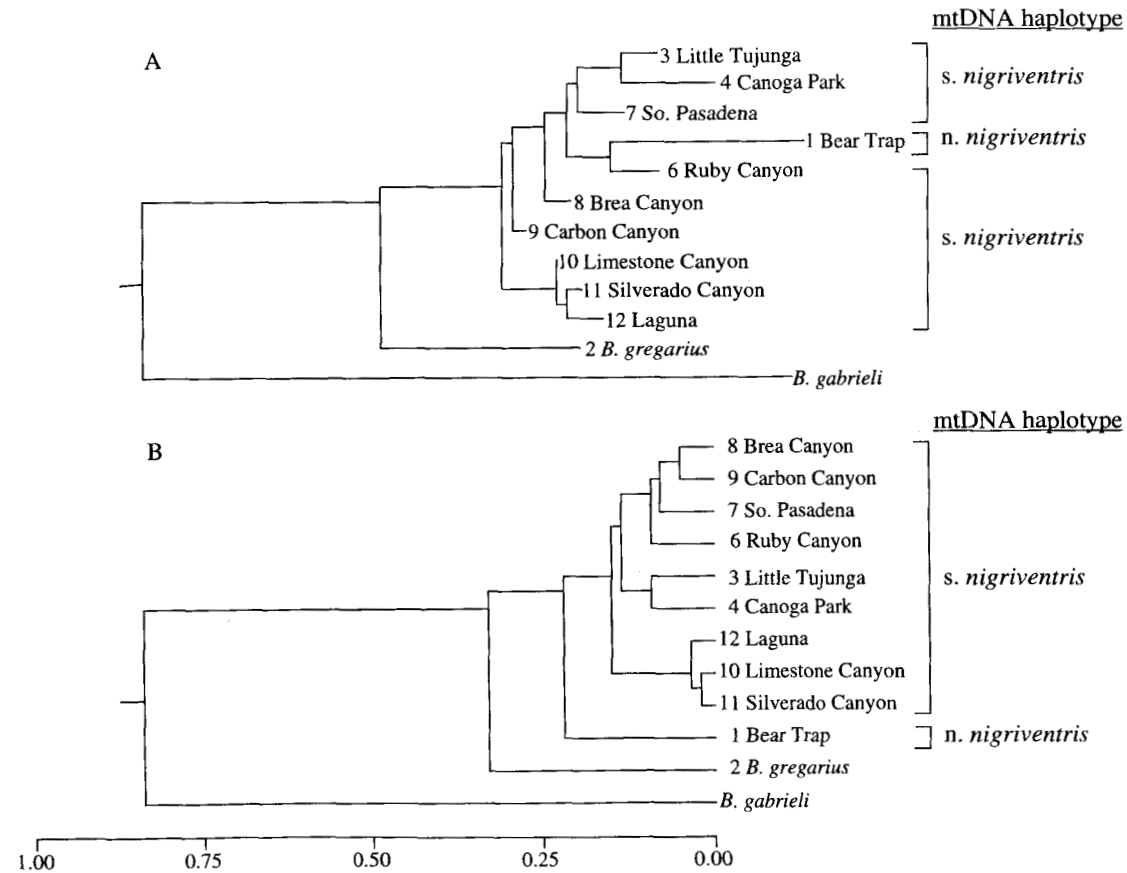

Figure 7. Neighbor-joining (A) and UPGMA (B) trees derived matrix of allozymic D_N (Table 6) for members of the *Batrachoseps nigriventris* complex in southern California, and outgroups. Column at right indicates mtDNA haplotype lineage.

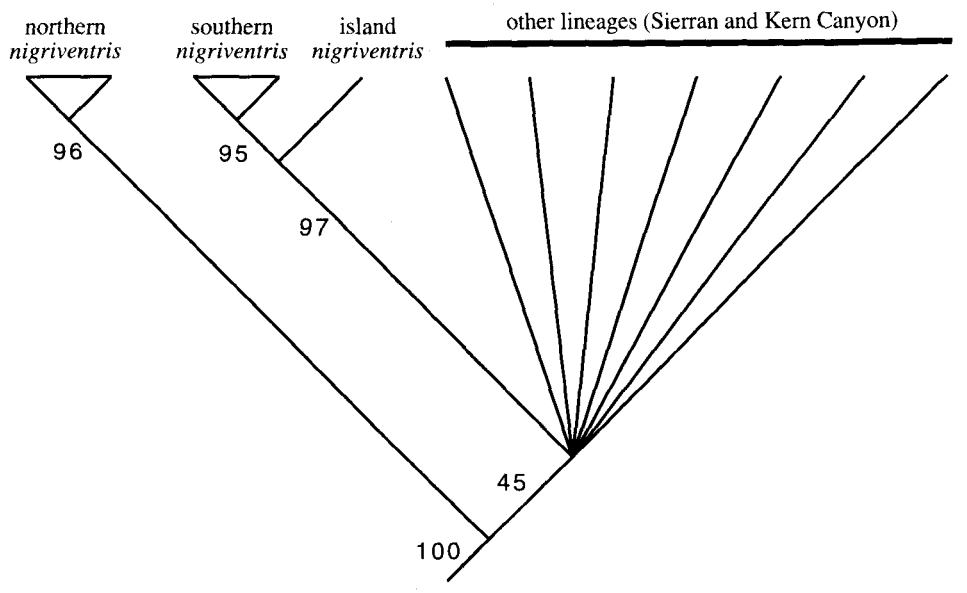

Figure 8. MtDNA phylogeny of the *nigriventris* complex, focusing on lineages currently included in *B. nigriventris*. Numbers along branches are percent of 100 bootstrap replicates in which that clade was supported (Jockusch and Wake, unpublished analyses).

Table 4. Samples used in allozyme studies of *Batrachoseps nigriventris* group in southern California. Numerals are the same as those in Figs. 6 and 7. Sample sizes indicated in parentheses. Study was conducted on 28 proteins, 26 of which were variable.

1. *Batrachoseps nigriventris* from between Bear Trap and Pastoria Canyons, Kern Co., Ca., 34°53.5′W, 118°45.2′W (n = 10).
2. *Batrachoseps gregarius* from ENE Lemon Cove, Tulare Co., Ca., 36°23.5′N, 118°59.7′W (n = 10).
3. *Batrachoseps nigriventris* from Little Tujunga Canyon, Los Angeles Co., Ca., 34°20.2′N, 118°20.2′W (n = 3).
4. *Batrachoseps nigriventris* from Canoga Park, Los Angeles Co., Ca., 34°13.3′N, 118°38.9′W (n = 4).
5. *Batrachoseps nigriventris* from Prisoner's Harbor, Santa Cruz Island, Santa Barbara Co., Ca., 34°01.0′N, 119°41.2′W (n = 20) (sample not used in this study; comparison with topotypic *B. nigriventris* in this paper based on Yanev, 1978).
6. *Batrachoseps nigriventris* from Ruby Canyon and upper Winter Creek trail, Los Angeles Co, Ca., 34°12.0′N, 118°01.3′W (n = 5).
7. *Batrachoseps nigriventris* from South Pasadena, Los Angeles Co., Ca, 34°06.8′N, 118°09.0′W (n = 10).
8. *Batrachoseps nigriventris* from Brea Canyon, Los Angeles Co., Ca., 33°58.0′N, 117°50.7′W (n = 10).
9. *Batrachoseps nigriventris* from Carbon Canyon, San Bernardino Co., Ca., 33°57.5′N, 117°45.5′W (n = 10).
10. *Batrachoseps nigriventris* from Limestone Canyon, Orange Co., Ca., 33°44.8′N, 117°40.7′W (n = 11).
11. *Batrachoseps nigriventris* from Silverado Canyon, Orange Co., Ca., 33°44.8′N, 117°37.0′W (n = 5).
12. *Batrachoseps nigriventris* from NE Laguna Beach, Orange Co., Ca., 33°33.7′N, 117°45.8′W (n = 7).

Additional out-group: *Batrachoseps gabrieli* from type locality, upper margins of canyon of N Fork San Gabriel River, Los Angeles Co., Ca. (n = 5) (from Table 1).

Table 5. Values of D_N between populations of the *Batrachoseps nigriventris* complex in southern California, as well as for two outgroups.

Population	1	2	3	4	5	6	7	8	9	10	11
1 Bear Trap	0.000										
2 *gregarius*	0.435	0.000									
3 Little Tujunga	0.237	0.360	0.000								
4 Canoga Park	0.285	0.363	0.083	0.000							
5 *gabrieli*	1.049	0.804	0.811	0.897	0.000						
6 Ruby & Anita	0.166	0.379	0.110	0.166	0.929	0.000					
7 So. Pasadena	0.221	0.344	0.089	0.121	0.826	0.072	0.000				
8 Brea Canyon	0.235	0.307	0.090	0.147	0.724	0.057	0.060	0.000			
9 Carbon Canyon	0.188	0.304	0.105	0.172	0.708	0.120	0.082	0.044	0.000		
10 Limestone	0.160	0.259	0.157	0.177	0.787	0.148	0.128	0.100	0.057	0.000	
11 Silverado	0.185	0.254	0.176	0.194	0.808	0.170	0.148	0.124	0.087	0.016	0.000
12 Laguna	0.183	0.341	0.185	0.195	0.905	0.175	0.139	0.145	0.089	0.033	0.035

The allozyme and mtDNA data offer conflicting patterns and suggest somewhat different histories for the *B. nigriventris* complex. In our haplotype tree (Fig. 8) the southern + island *nigriventris* clade is more closely related to other members of the *nigriventris* lineage than to northern *nigriventris*. By contrast, in the allozyme UPGMA tree (Fig. 7) southern *nigriventris* clusters with northern *nigriventris*. Other members of the *nigriventris* group are more basal, first *gregarius*, then even more basally *stebbinsi* and *simatus* (results for last two species based on unpublished data, not shown). Yanev's (1980) analysis included no southern *nigriventris*, but she had a large number of samples of *gregarius* and northern *nigriventris* and they clustered with each other to the exclusion of both *simatus* and *stebbinsi*. Our sample of *gregarius* is less differentiated from southern *nigriventris* ($D_N = 0.25–0.38$, $\bar{x} = 0.29$; it differs from southern *nigriventris* by fixed differences at one locus and nearly fixed differences at three loci) than it is from northern *nigriventris* ($D_N = 0.44$ to topotypic material), but southern *nigriventris* is even less differentiated from northern *nigriventris* than from *gregarius* ($D_N = 0.16–0.29$, $\bar{x} = 0.21$). These inconsistencies in the patterns of relationships suggested by different data sets may be taken as a suggestion that genetic admixtures have occurred. Additional

Table 6. Divergence between mtDNA haplotype groups in the *nigriventris* complex of *Batrachoseps* in southern California. Data as in Table 3. Northern *nigriventris* includes topotypic *nigriventris*. Other includes populations from the Sierra Nevada and adjacent mountains currently included in *B. gregarius*, *B. simatus*, *B. stebbinsi*, and additional populations of uncertain taxonomic status from the Kern Canyon region of California.

	Northern *nigriventris*	Southern *nigriventris*	Island *nigriventris*	Other
Northern *nigriventris*	0.059 0.19[1]	0.052–0.115	0.052–0.091	0.056–0.126
Southern *nigriventris*	0.16–0.29	0.034 0.20	0.0034–0.041	0.051–0.109
Island *nigriventris*	0.17	n.a.	— —	0.046–0.081
Other	0.16–1.04[1]	0.25–0.39[2]	0.41–0.75[1]	0.078 0.94[1]

[1]Data from Yanev (1978).
[2]Comparisons are only available to *B. gregarius*.

support for this possibility comes from alternative methods of analyses of the allozyme data (NJ, Fig. 7; Wagner clustering, phylogenetic analysis of allele coding, not shown) in which the single northern *nigriventris* sample always falls within the southern *nigriventris* cluster. An alternative explanation for the apparent conflict is that we have failed to recover the true history of one or both markers. The position of northern *nigriventris* as the sister to the rest of the *nigriventris* group is only weakly supported in phylogenetic analyses of the mtDNA data. The bootstrap value for this node is only 45% (Fig. 8). In analysis of only the *nigriventris* complex, trees in which northern *nigriventris* is constrained to be the sister to southern and island *nigriventris* are only four steps (0.6%) longer than the most parsimonious trees, and these trees are not significantly worse by the Templeton test ($P = 0.48$–0.51).

Even if *nigriventris* proves to be monophyletic with respect to mtDNA, this will not resolve the taxonomic dilemma because of the deep divergences within the complex and the unlikeliness of genetic exchanges between the parts. If the conflict between the mtDNA and allozyme analyses is the result of genetic admixture, recognition of a single species in southern California, *nigriventris* as currently constituted, might be appropriate. Arguments for recognition of the island population as a distinct species would still apply however, and are essentially the same as used for recognition of island populations of the *pacificus* group (except that island *nigriventris* is less distinct morphologically from mainland relatives than is the case for *pacificus*, Brame and Murray, 1968). Furthermore, the presence of fixed allelic differences and a concordant break in mtDNA argue in favor of breaking mainland *nigriventris* into two species. There are two fixed allozymic differences between northern and southern *nigriventris* (for isocitrate dehydrogenase-2 and superoxidase dismutase), and there is one fixed difference (for L-iditol dehydrogenase) between northern *nigriventris* and island *nigriventris* (although we did not compare island populations with southern *nigriventris*, northern and southern *nigriventris* are similar with respect to this protein so we expect at least one fixed difference between southern *nigriventris* and island populations). The taxonomic status of the two clusters of mainland *nigriventris* depends on analysis of their zone of contact. Our sampling for mtDNA is relatively complete. Northern and southern *nigriventris* (identified by haplotypes) have been found within about 30 km of each other. There is a geographic gap of about 90 km in our allozyme sampling. D_N between near topotypic *nigriventris* and the closest population of southern *nigriventris* is 0.22 (populations 1 to 3, Fig. 4), and the value to the next closest population of southern *nigriventris* is even greater, 0.29. We consider it unlikely that additional sampling in intervening areas will close such a large genetic gap, in part because salamander populations are very sparse in this region.

While there are no evident geographic barriers between northern and southern *nigriventris*, most of the intervening area is unsuitable for salamanders. Furthermore, northern and southern *nigriventris* are separated by two of the largest fault systems in western North America, the NW-SE trending San Andreas Fault system and the crossing NE-SW trending Garlock Fault system. The region is geologically unstable, with land masses having undergone major displacements with respect to each other during the past few millions of years (Atwater, 1989). These factors may have served to isolate the two phylogeographic units in the *nigriventris* complex that we have identified.

For the present, *nigriventris* contains three genetically distinct groups: (1) northern and western populations that are close relatives of topotypic populations in the Tehachapi Mountains; (2) Santa Cruz Island populations; (3) southern California populations that extend from Sierra Pelona (near Bouquet Reservoir) and more western

mountain areas in the vicinity of Pyramid Lake and Lake Piru, through the Santa Susanna, Santa Monica and San Gabriel Mountains, and the Baldwin Hills, Chino Hills and Santa Ana Mountains, to the southern borders of Orange and Riverside counties. In general, the last group occurs in uplands or at least low hills in southern California, not in the low-lying valleys, with the exception of the southern-most locality along the coast in South Laguna Beach, Orange County. Pending additional research, we suggest the following taxonomy for the populations that Yanev (1980) included in *Batrachoseps nigriventris*:

3.2.2.1. *Batrachoseps nigriventris* Cope 1869. Distribution: From extreme southern Monterey County where it occurs from the Pacific Coast inland to western Fresno County and south through the coast ranges and associated valleys to southern Kern (western and central Tehachapi Mountains) and central Ventura counties. From northwestern Los Angeles County in upland areas to southern Orange and southwestern Riverside counties; widely distributed on Santa Cruz Island. Occurs from near sea level to about 2500 m (on Mt. Pinos).

3.2.2.2. *Batrachoseps gregarius* Jockusch, Wake, and Yanev 1998. Distribution: From the southern boundary of Yosemite National Park south nearly to the Kern River on the west slope of the Sierra Nevada and in the western Greenhorn Mountains. Ranges in elevation from below 300 m to about 1800 m.

4. CONCLUSIONS

Taxonomic resolution of such complicated patterns of relationships as occur in the genera *Ensatina* and *Batrachoseps* necessarily involves compromises. The Linnean taxonomy presently in use is too inflexible to express adequately the conflicting patterns of character data that we have outlined. The three species complexes that we have discussed are relatively old (judging from degree of molecular divergence), and they have had a long time in which to lose cohesion. However, varying degrees of cohesion remain, as evidenced by the nature of population interactions following recontact. Each presents a unique set of complicating factors, leading us to propose three different solutions. In the case of *Ensatina*, the dynamics of differentiation are captured neither by the classical polytypic species taxonomy employed by Stebbins (1949) (and still recommended as a default taxonomy by Wake and Schneider, 1998), nor by a taxonomy that would break up the taxon into many species (Highton, 1998). In the case of *Batrachoseps*, some species are non-controversial because they occur in sympatry and are well differentiated genetically (Jockusch, 1996; Yanev, 1980), but because they are so difficult to separate using morphological traits even sympatric members of different species groups were confused until recently (e.g., *major* and *nigriventris* in southern California were often considered to be conspecific until Brame and Murray, 1968). However, allopatric populations have always posed problems for systematists. Molecular data often clarify such situations, but they can also complicate matters. With respect to *Batrachoseps* in southern California, we interpret the molecular data to suggest that cohesion remains in *B. major* but may not in *B. nigriventris*. We believe these cases provide insight into the nature of problems that we are increasingly likely to confront in future systematic studies.

5. SUMMARY

Many of the plethodontid salamanders in western North America have been studied for variation in allozymes and mtDNA sequences. Often results from these two data sets are concordant with each other and with morphology, and they help define species borders. However, there are instances in which the units defined by one data set do not coincide with those based on another data set. What appear to be species from the perspective of one data set might best be considered a phylogeographic segment of a larger interbreeding unit from the perspective of the combined data. Examples are given for the *Ensatina* and *Batrachoseps* complexes, both of which present daunting taxonomic dilemmas. In northern California and the Sierra Nevada, discordance in *Ensatina* is interpreted to be the result of periods of isolation and associated genetic fragmentation, followed by periods of differential admixture of genes. In *Batrachoseps* in southern California, two different patterns of non-concordance of allozyme and mtDNA data are encountered. In the *pacificus* complex, highly divergent mtDNA haplotype clades persist in the face of widespread admixture of nuclear genes. We recommend elevating *major* and *pacificus* to the status of full species, in order to reflect their long independent evolutionary history, and reducing *aridus* to the status of a subspecies of *major*, because we believe that recent gene flow has partly reunited once separated units. In the *nigriventris* complex, mtDNA and allozyme data identify the same three major lineages in southern California; however, they indicate different genealogical relationships for these lineages, suggesting that gene flow between differentiated groups may have occurred in the past. Complete taxonomic resolution for this group depends critically on analysis of the contact zone, and may result in the description of additional species. The three cases studied illustrate the difficulty in making taxonomic decisions even when much data are available.

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